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Report Title

Final Report on "Generation of Advanced Diagnostics and Countermeasures for Individuals Most Vulnerable to Biothreats". Proposal #42299LS. Funding Number: DAAD190110450

ABSTRACT

In the first phase of this proposal, we identified mouse strains differentially resistant and susceptible to infection with three biothreat pathogens, Cowpox, Influenza, and Anthrax. In the second phase, the scope of our project is to identify: 1. Host-Oriented Pathogen Response (HOPR) Signaling Pathways, as the underlying mechanism responsible for host-specific susceptibility; and 2. the specific protein constellations responsible for the resistant or susceptible phenotypes. Pinpointing the specific protein(s) responsible for the host responses will allow us to develop specific drugs to convert susceptible into resistant phenotypes. Our ultimate goal is to use the knowledge and enabling technology gained for advanced diagnosis and countermeasures to biothreats.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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Number of Inventions:

Graduate Students

NAME

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FTE Equivalent:

Total Number:

Names of Post Doctorates

NAME

PERCENT SUPPORTED

FTE Equivalent:

Total Number:

Names of Faculty Supported

NAME

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FTE Equivalent:

Total Number:

Names of Under Graduate students supported

NAME

PERCENT SUPPORTED

FTE Equivalent:

Total Number:

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

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Names of other research staff

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FINAL REPORT (SEPTEMBER 23, 2006)

Project Title: *Generating Advanced Diagnostics and Countermeasures for Individuals most Vulnerable to Biothreats*

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Summary of the Scope of the Project: In the first phase of this proposal, we identified mouse strains differentially resistant and susceptible to infection with three biothreat pathogens, Cowpox, Influenza, and Anthrax. In the second phase, the scope of our project is to identify: **1. *Host-Oriented Pathogen Response (HOPR) Signaling Pathways***, as the underlying mechanism responsible for host-specific susceptibility; and **2. the *specific protein constellations*** responsible for the resistant or susceptible phenotypes. Pinpointing the specific protein(s) responsible for the host responses will allow us to develop specific drugs to convert susceptible into resistant phenotypes. Our ultimate goal is to use the knowledge and enabling technology gained for advance diagnosis and countermeasures to biothreats.

Accomplishments To Date in Phases 1 and 2:

Phase 1: Phase 1 is composed of years 1 and 2 for this project, during which we identified recombinant inbred mouse strains resistant or susceptible to biowarfare (BW) pathogens, as illustrated in Table 1. In addition, we identified: **1.** Constitutive host-oriented oxidative defenses, as the key to protecting hosts from early pathogen infection-associated insults, *i.e.* the first 24 hours post-infection; and **2.** by microarray study, that GST, BRCA and their sister genes' expressions can be used as biomarkers for Host Vulnerability.

Genetic responses, in terms of anti-oxidant response (ARE) and transcriptional factor (E-box) genes, to cowpox virus infection were analyzed by microarray gene screening assays, for differential gene expression between cowpox-susceptible C57 and -resistant C3H mice. Lung tissue RNA of mice sacrificed 1 and 6 days after cowpox virus (CPV) inoculation were compared with samples from uninfected mice, using a customized microarray system; the results were validated by Real-Time PCR. On E-box gene chips, more than 20 genes are up-regulated after inoculation in both strains (Please see Table attached.). The genes react to the insult promptly and evidently on day 1, and then reach much higher levels by day 6. 7 E-box genes are significantly up-regulated: Brac1, Odc, lef1, Chrng, zfp263, NeuroD and Tcfec. Tp53 exhibits differential response, up-regulated in the resistant C3H strain, and down-regulated in the susceptible C57 strain. Significant down-regulation of Nos2 is observed in both C57 and C3H strains, implying serious injury in the lungs of infected mice. Among ARE genes, GST responds very quickly and strongly, reaching a very high level on day 1, but subsiding by day 6. Keap1 shows significant up-regulation too, but only in resistant C3H mice. Odc, Brac, Trp3 and Nos2, as well as GST and Keap1, are useful biomarkers for monitoring cowpox infection. Tp53 and GST are expressed much higher in C3H than C57 mice, revealing that they are important genetic factors protecting C3H against cowpox insult. Pathway analysis for E-box and ARE genes participating in the cellular response to CPV insult, potential mechanisms of protective function, reveals that proinflammatory and anti-oxidant signaling are involved.

Phase 2: Phase 2 is composed of the 3rd and 4th years of this proposal; we have expanded beyond microarray screening to proteomic profiling as the enabling high-throughput technology to generate host res-

Table 1. Results of the First Titration Experiments with both Male and Female Mice

Mouse Strains Pathogens	C57 / Black	129	Balb / c	DBA	C3H
<i>R. conorii</i>	A	A	B	C	C
VEE	C	C	C	C	C
West Nile Virus	C	C	C	C	C
Cowpox	C	B	B	B	A
Influenza	A	C	A	C	C
Anthrax	D	D	D	D	D

Coding:

A. Resistant at all doses used

B. Transiently ill, all recover

C. Dose-dependent lethal (with LD₅₀ varying among strains)

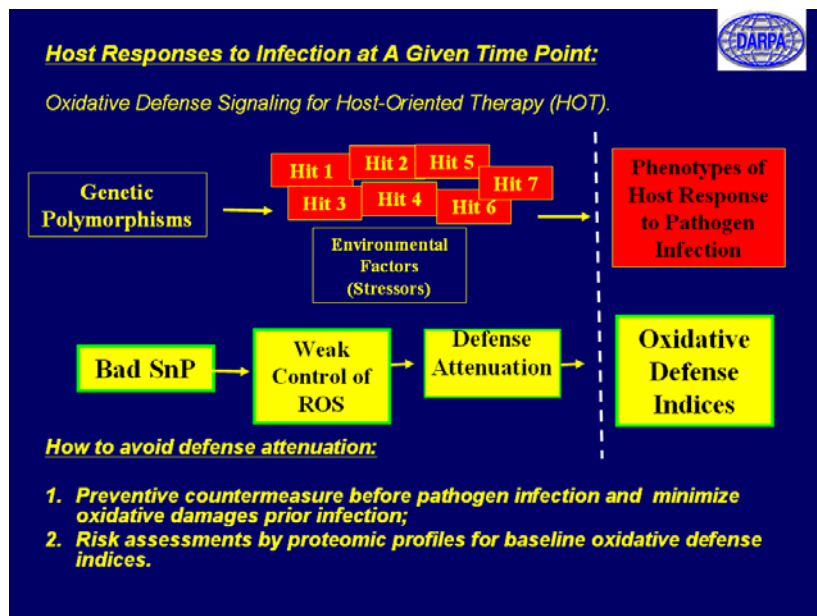
D. Extremely potent; all animals die within three days

ponse protein signatures to biothreat pathogens, with the ultimate goal of identifying protein constellations responsible for host resistance or susceptibility. Our focused model system is three recombinant inbred mouse strains, C57/B, C3H, and Balb/C, infected with three pathogens, Cowpox, Flu, & Anthrax. Using high-throughput Liquid Chromatography-Tandem Mass Spectroscopy with the QStar set-up, we obtained significant separation of all labeled proteins from HPLC fractions. Peptides from these fractions were then profiled; 252 proteins were identified as differing between the total cell protein pools of the lung tissues of the three different mouse strains. Among them, more proteins show differences comparing C3H and C57 than Balb/C and C57. This confirms our animal studies, which show that C3H is susceptible, while both Balb/C and C57 are similar in that both are resistant strains.

Out of the 252 proteins identified as differing among the three mouse strains, the confidence level for at least 210 proteins is above 90. This is very high, indicating scant possibility that results obtained are due to false positives, a comfort level required for large-scale proteomic profiling, as well as reducing effort later on to validate the results. As discussed below, peroxiredoxin 6 exhibits 35- and 40-fold differences comparing C3H and Balb/C, respectively, with C57. We validated this result by two-dimensional (2-D) gel proteomics.

Among the identified unique protein signatures exhibiting significant fold changes are hemoglobin beta chain, immunoglobulin gamma-2A, chaperonin, apolipoproteins, Sal-like protein 1 (Sal 3), and peroxiredoxin 6. Besides peroxiredoxin 6, described above and known to be functionally involved in oxidative defense, of the remaining signature proteins identified, the most interesting is apolipoproteins, related to age-dependent diseases such as cardiovascular and neurological disorders. Identifying this protein difference at the baseline uninfected state may reflect genetic differences among these mouse strains in their risk level for these disorders.

Interestingly, all proteins exhibiting significant fold changes pertain to stress-associated signaling



pathways. Functionally, they are involved in the mechanisms of host responses to oxidative stress, and reflect the genetic diversity among the three mouse strains in terms of heritable traits in dealing with environmental stress; pathogen infection is one of these stressors. This underlying genetic difference may dictate the resistant or susceptible phenotype of various mouse strains.

In conclusion, results of Phase 1 by genetic and microarray studies, and phase 2 by proteomic profiling, with the recombinant inbred mouse strains show that each mouse strain has its own response profile, with unique signature gene expression patterns. These results support our suggestion that host-oriented pathogen signaling pathways are in-

trinsically determined by genetic and environmental factors, represented as “Hit #” in red in the above figure, and that together these two families of factors eventually define host resistant or susceptible phenotypes to biothreat pathogen infection. Moreover, we suggest that **oxidative defense indices (ODI)** can be the combined effect of: 1. bad genetic factors, denoted commonly as bad single nucleotide polymorphic features (Bad SNP); and 2. environmental hits rendering hosts weak in control of reactive oxygen species (ROS), the free radicals. The combined forces of “bad SNP” and “Weak Control of ROS”, as shown in the above figure, eventually cause attenuation of host defense against pathogen infection. Future work will quantify this attenuation, generate oxidative defense indices, and identify advance diagnostics to predict whether a host is resistant or susceptible, prior to infection ever occurring.